Datasheet for ABIN2477109

anti-Vitronectin antibody

1 Validation

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Overview

Quantity:	25 µL
Target:	Vitronectin (VTN)
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Vitronectin antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (Frozen Sections) (IHC (fro))

Product Details

Immunogen:	Purified vitronectin from human plasma
Clone:	8E6 (1-110)
Isotype:	IgG1
Characteristics:	Purified IgG
Purification:	Purified

Target Details

Target:	Vitronectin (VTN)
Alternative Name:	Vitronectin (VTN Products)
Gene ID:	7448
UniProt:	P04004

Target Details

Pathways:	Autophagy, Smooth Muscle Cell Migration

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.0 mg/mL

Successfully validated (Flow Cytometry (FACS))

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Report Number: 103145

Date: Aug 02 2018

Target:	VTN
Lot Number:	300615
Method validated:	Flow Cytometry (FACS)
Positive Control:	Melanoma cell line Ma-Mel-86B endogenously express Vitronectin Freshly isolated monocytes were transfected with human Vitronectin (VTN) full length cDNAs in pcDNA3.1 vector
Negative Control:	293T cells, AML cells, HUVEC cells, MRC-5 cells, CAF cells
Notes:	ABIN2477109 specifically recognizes Vitronectin in primary monocytes transiently transfected with a full-length VTN expression vector as well as in Ma-Mel86B positive cell line.
Primary Antibody:	ABIN2477109
Secondary Antibody:	ABIN4323449
Isotype:	IgG1 isotype control antibody (Beckmann Coulter, product no IM0571, lot 35)
Protocol:	• Isolate monocytes from healthy donor from PBMCsprepared by Ficoll-Hypaque density gradient and enriched by plastic adherence in 3ml volume on a 6 well-platefor 90min at 37°C in RPMI (Gibco, 21875-034, lot 1926384) supplemented with 1.5% of human serum (pooled serum from healthy donors). Cultivate adherent cells for 24h in RPMIsupplemented with 10% human serum and PenStrep (Sigma, P0781, lot 077M4843V), at 37°C and 5% CO_2 in 3ml volume on a 6 well-plate to 60-70% confluency. • Cultivate 293T cells, AML cells and Ma-Mel86B cells in RPMI (Gibco, 21875-034, lot 1926384) supplemented with FCS (Sigma, F7524, lot BCBS0318V) and PenStrep (Sigma, P0781, lot 077M4843V) at 37°C and 5% CO_2 in cell culture bottles or in petri-dishes to 60-70% confluency. • Cultivate MRC-5 cells in RPMI supplemented with 20% FCS and 1% PenStrep. • Cultivate HUVEC cells in EGM-2 (Lonza) supplemented with 1% PenStrep. • Cultivate CAF cells (MEM Eagle 10% FCS, 1% PenStrep, Na-Pyruvate, non essential amino acids, L-Glutamin). • Transfect cells with 300ng of the pcDNA3.1-human VTN-cDNA construct using Lipofectamin 2000 (Invitrogen, 11668-019, lot 1641316) following the manufacturer´s instructions. • Wash cells with FACS buffer.

- Centrifuge cells at 500xg for 5min at RT, aspirate the supernatant.
- Resuspend cells in 2ml ice-cold FACS buffer (PBS, 0.1% BSA, 2mM EDTA).
- Wash the cells 1x with FACS buffer and centrifuge them for 500xg for 5min at RT.
- Primary antibody:
  - Prepare three different dilutions (1:20, 1:100 and 1:200) of the mouse anti-human VTN antibody (antibodies-online, ABIN2477109, lot 300615) in FACS buffer. Add 50µl of each antibody dilution directly on the cell pellet and incubate for 30min at 4°C.
  - Resuspend the cell pellet with 100µl of FACS buffer with IgG1 isotype control antibody (Beckmann Coulter, product no IM0571, lot 35). Add 5µl directly on the pellet and incubate for 30min at 4°C.
- Wash cells 1x with FACS buffer and centrifuge them for 500xg for 5 min at RT.
- Resuspend the cell pellet with 50µl of FACS buffer with in secondary Goat- anti-mouse FITC antibody (antibodies-online, ABIN4323449, lot 132660) diluted 1:50 in FACS buffer and incubate for 30min at 4°C.
- Wash cells 1x with FACS buffer and centrifuge them for 500xg for 5min at RT.
- Resuspend pellet in 200µl 1% Paraformaldehyde solution to fix cells.
- Data acquisition in a BD FACSCanto II flow cytometer using Blue laser 488nm and filter 530/30.

Experimental Notes:

- Total RNA was isolated from different cell lines and primary cells and transcribed into cDNA. The full-length VTN ORF was amplified by PCR with specific primers and the PCR product was sequence-verified. PCR-positive Ma-Mel-86B cells served as positive control. Freshly isolated monocytes, MUTZ-3 cells, human primary AML cells and 293T cells served as negative controls.
- ABIN2477109 (1:20 dilution) reveals a specific fluorescence signal in Ma-Mel-86B positive control cells in 60% of the registered events. Although freshly isolated monocytes were negative by PCR, they show 21% cell surface expression of Vitronectin by FACS staining (low surface expression defined by a shift of the total cell population).
- The percentage of VTN positive cells could not be increased by transient transfection of monocytes with pcDNA3.1-human VTN-cDNA construct. Nevertheless, it seems that the surface expression of Vitronectin in the VTN positive population is higher and more concentrated to a part of cell population. This might indicate that ABIN2477109 specifically bind to VTN expressed on the transfected cells.
- No signal was registered in different primary cells: MRC-5 (primary fibroblast cells), HUVEC (primary endothelial cells) and CAF (cancer associated fibroblast cells).
- The antibody titration experiment reveals that the optimal dilution of the antibody for FACS staining was 1:20. Due to the small volume (25µl) of antibody provided, we did not have enough material to test other cells lines/primary cells.
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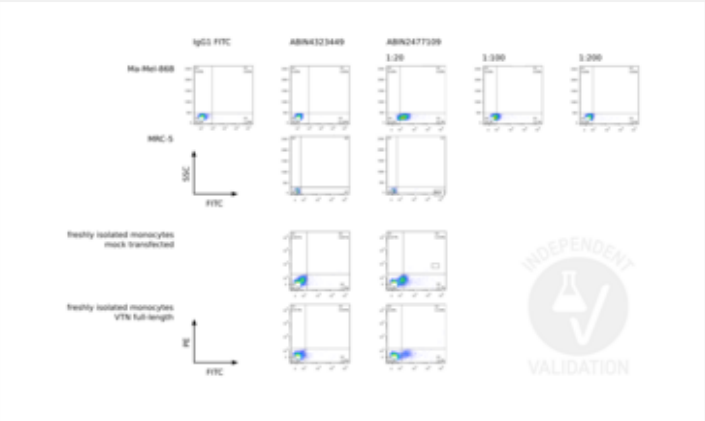

Validation image no. 1 for anti-Vitronectin (VTN) antibody (ABIN2477109)

Flow cytometry analysis of melanoma cell line Ma-Mel-86B endogenously expressing VTN, primary fibroblast cell line MRC-5, and freshly isolated monocytes with or without transfection of a VTN expression plasmid subsequently to incubation with the primary VTN antibody ABIN2477109 and the secondary, FITC-labeled antibody ABIN4323449.